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Results of a Twenty-Two Year Old Progeny Test of a *Pinus sylvestris* Plantation in Eastern Nebraska

Cover Page Footnote

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1. Introduction

Scots pine (*Pinus sylvestris* L.) is one of the most widely distributed pine species in European and Central Asian boreal, temperate, and sub-alpine forests. Scots pine extends from the Scottish Highlands along the Atlantic, to the Pacific coast of Siberia, and ranges in elevation from sea level to an altitude of 2600 meters (Richardson 1998). The northeastern limit of the species in Siberia probably reflects its inability to withstand cool summers and extremely cold winters (Vakovski 1958 as cited in Richardson 1998).

Like several other pine species, Scots pine has had significant interactions with humans throughout history. Some interactions have had positive effects on the species, such as replanting and limiting grazing activities in areas, while others have had damaging interactions, such as limited regeneration due to heavy grazing and use as firewood between the 15th and 18th century for iron smelting (Bonhote & Vernot 1988 as cited in Richardson 1998). This over-utilization resulted in the disappearance of Scots pine forests from areas in Britain, Ireland and Denmark (Mirov 1967, Kinloch et al. 1986 as cited in Richardson 1998).

Scots pine has been planted in the United States for over 200 years (Van Haverbeke 1986). It was first introduced for uses such as windbreaks to protect crops, animals, and properties especially during winter times; to prevent soil erosion; to serve as habitat for wildlife; to reforest coal mine spoils; and for aesthetic purposes (Vogel 1981).

New disease problems, primarily the Pine Wood Nematode (PWN, *Bursaphelenchus xylophilus*), are now limiting the use of Scots pine to Christmas trees. The PWN was first reported in North America in 1979 (Dwinell 1997). It is now known to be the causal agent of Pine Wilt Disease, a lethal disease of Scots pine and other introduced pine species in the eastern and central United States. For example, PWN is reported to have killed thousands of Scots pine trees during the 2001 to 2003 drought in Kansas (James et al. 2006).

In the Great Plains, ponderosa pine (*Pinus ponderosa* P&C Lawson) is the only major native pine species; therefore, several non-native pines originating from different seed sources (provenances) have been introduced and planted throughout the area. In 1962, a Scots pine plantation originating from 36 origins across Europe and Asia was established at Horning State Farm near Plattsmouth, Nebraska. This was part of the Regional Tree Improvement Project (NC-99, formerly NC-51) of the North Central State Agricultural Experiment Stations (Read 1971). In 1970 each tree was rated for foliage color, balance, foliage density, form, taper, needle retention and stem straightness; all desirable characteristics for

Christmas and ornamental tree selection. Forty-three superior phenotypes were selected as the breeding population for establishing a clonal seed orchard and progeny test (Van Haverbeke 1976). Read (1971), reported the eight-year results of the 1962 (36 provenance) planting. In the spring of 1971, a seed orchard was established at the University of Nebraska Agricultural Research and Development Center (ARDC) near Mead, Nebraska, by planting 3 year-old stock of 18 of the original 36 provenances (Van Haverbeke 1974, Van Haverbeke 1976). Van Haverbeke (1986) reported the twenty-year results of the seed orchard. Concurrently, the beginning of a full-sib progeny test was established with controlled matings of the selected trees (Van Haverbeke 1976.). The purpose of this study is to test the performance and adaptability of the twenty-two-year-old *Pinus sylvestris* full-sib progeny plantation to the Great Plains environment.

2. Materials and Methods

Site characteristics and origin of plantation

The study was located at the University of Nebraska Agricultural Research and Development Center (ARDC), Mead, Nebraska (366 m altitude, 41° 08' latitude and 96° 30' longitude). The long-term (1961 to 2001) annual precipitation averages 741 mm with about 75% falling during the growing season (HPRCC, 2001). The average annual temperature on site is 10.5 °C. The average temperature in January is -6 °C and the average temperature in July is 25°C. The soil types of the site are Filbert silt loams (fine, smectitic, mesic Vertic Argialbolls) and Tomek silt loams (fine, smectitic, mesic Pachic Argiudolls).

The concurrently established breeding program began at the same time as the seed-orchard because the uniform environment of the test site made it more likely that the phenotypic expressions of the selected trees were probably the result of their genotypic potential. It was also reasoned that several years time could be saved by running both programs at the same time (Van Haverbeke 1976). Each selected tree was mated with seven pollen donors (tester trees representing six provenances) from among the selected trees (Van Haverbeke 1979, Van Haverbeke 1976). Due to the young age of the trees (selected at age of eight), tester trees were chosen based on the presence and quantity of strobili (Van Haverbeke 1976). The “Mini Bag” technique and plastic bottle pollinators were used for making the controlled pollinations and to prevent wind pollination (Van Haverbeke 1976, Van Haverbeke 1973). Seed for the test was sown in the spring

of 1979 in the greenhouse and outplanted at the ARDC site as 2-0 seedlings in 1981 (Van Haverbeke 1979). A total of 23 crosses were created and planted at the ARDC. Seedlings were planted in a randomized complete block design at a spacing of 2 x 4 m (within and between rows, respectively). The understory herbaceous vegetation was controlled to reduce competition for resources by either chemical application (herbicide) or grazing.

The performance of the twenty-two-year-old progeny plantation was evaluated in 2002. The plantation represented crosses of genetic material from seven European countries [Spain (SP), France (FR), Russia (RUS), Ex-Yugoslavia (YOG), Italy (IT), Greece (GR), and Germany (GER), Figure 1]. Paternal genetic material originated from IT, YOG, FR, RUS and GR, and, maternal genetic material originated from IT, SP, FR, GR, YOG, RUS, and GER.

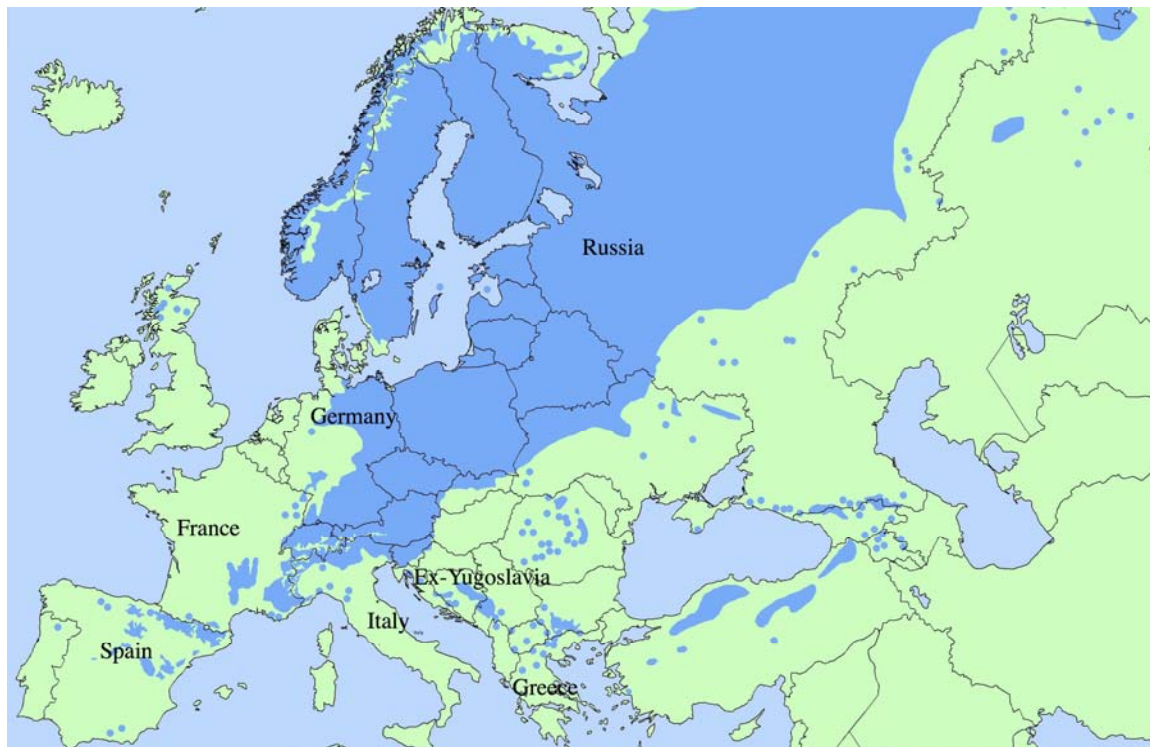


Figure 1. Sources of genetic material for *Pinus sylvestris* progeny test plantation at the University of Nebraska Agricultural Research and Development Center near Mead, Nebraska and range of *Pinus sylvestris* in Europe (Modified from Mátyás et. al 2004).

Sampling procedure

A total of 92 individuals originating from 23 crosses were randomly selected for the study. The following growth and vigor parameters were determined: specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$), the needle length (cm), canopy width (m), and tree height (m). Specific leaf area was estimated using needles randomly selected from bottom, middle and upper canopy of each tree. Leaf area was determined using a portable leaf area meter (*LI-300 LI-COR Inc. Lincoln NE, USA*). Needles were then dried in a forced-air oven at 70°C until weight was constant and specific leaf area ($\text{SLA} = \text{leaf area} / \text{dry weight}, \text{cm}^2 \text{g}^{-1}$) was subsequently determined. Needle length was determined on needles sampled for SLA using a ruler. Canopy width at its widest point was determined with a measuring tape in both north/south and east/west directions and averaged on a tree basis for the statistical analysis. Tree height was measured with an Abney hand level (Hush et al. 1993).

Statistical Analyses

Data were analyzed using the JMP 5.0.1 statistical package. Analysis of variance and orthogonal contrasts were used to compare crosses. Principal component analysis (PCA) was used to understand the factors controlling seed source classification.

3. Results

Height, which is considered an important indicator variable (Avery and Burkhart 2002) significantly differed between seed sources at all levels (paternal, maternal, and individuals) (Table 1). Orthogonal contrasts were used to compare trends and showed that there was a significant difference ($P=0.0001$) between maternal sources from southern (GR+IT+SP+FR) and northern (GER+RUS+YOG) countries and a significant difference ($P=0.0001$) between maternal sources from southern and northern countries and this pattern held whether France was considered northern or southern (Table 2). There were also significant differences ($P=0.0001$) between paternal sources from (GR+IT) and (RUS+YOG+FR); (GR+IT) and (FR); and (GR+IT) and (RUS+YOG) (Table 2). On average, paternal sources originating from RUS and GR were taller (12.7 m) than the remaining sources (IT, YOG, and FR, average 11.7 m) (Figure 2, Table 3). Maternal sources originating from RUS and GER were taller on average (13.1 m) than those from FR, GR, and YOG (12.21 m) and IT and SP (11.5 m) (Fig. 2,

Table 3). Height ranged between 10.68 m (FR x IT) and 14.13 m (GR x RUS) (Table 3).

Canopy width was found to be statistically significant at both paternal ($P=0.036$) and maternal ($P=0.015$) levels (Table 1). Orthogonal contrasts were used to compare trends and showed that there was a significant difference ($P=0.023$) between maternal sources from southern and northern countries and this pattern held whether France was considered northern or southern (Table 2). Canopy width in trees from paternal sources originating from GR (4.18 m) did not differ from those originated from RUS (4 m) but were significantly wider than those from FR, YOG, and IT (average 3.58 m) (Figure 2). Average canopy width of maternal sources originating from GER (5.5 m) was wider than GR, FR, RUS, YOG, IT, and SP (average 3.7 m) (Figure 3). Canopy width ranged from 2.75 m (YOG x RUS) to 5.5 m (IT x GER) (Table 3).

Needle length and specific leaf area (SLA) are used as indicator variables to predict productivity through biomass production and adaptation to environmental conditions. Needle length differences were found to be statistically significant only at the paternal level ($P<0.0001$) (Table 1). Orthogonal contrasts were used to compare trends and showed that there was a significant difference ($P=0.0001$) between all paternal sources from (GR+IT+FR) and (RUS+YOG); (GR+IT) and (RUS+YOG+FR); (GR+IT) and (FR); and (GR+IT) and (RUS+YOG) (Table 2). Average needle length from paternal seed sources originating from RUS and YOG were longer (7.38 cm) than those from GR (7.02 cm), IT (6.49 cm), and FR (6.04 cm) (Figure 2). Average needle length in maternal sources from RUS and GER were longer (7.75 cm) than those from FR, IT, GR, and YOG (average 6.59 cm) and IT (6.44 cm) (Figure 3). Needle Length ranged from 5.45 cm (FR x SP) to 8.91 cm (YOG x RUS) (Table 3).

Table 1 and table 2 show that SLA differences were not statistically significant at $P<0.05$. Average SLA for paternal sources was $19.28 \text{ cm}^2 \text{ g}^{-1}$, regardless of origin (Figure 2). However, Figure 3 shows differences in average SLA between maternal sources. Average SLA of maternal sources from FR ($22.42 \text{ cm}^2 \text{ g}^{-1}$) was greater than those from RUS, IT, GR, and YOG ($19.18 \text{ cm}^2 \text{ g}^{-1}$), GER ($16.18 \text{ cm}^2 \text{ g}^{-1}$), and SP ($12.15 \text{ cm}^2 \text{ g}^{-1}$). SLA ranged from $12.15 \text{ cm}^2 \text{ g}^{-1}$ (FR x SP) to $28.46 \text{ cm}^2 \text{ g}^{-1}$ (IT x FR) (Table 3).

Table 1. Analysis of variance table for specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$), needle length (cm), canopy width (m) and tree height in a Scots pine (*Pinus sylvestris*) progeny plantation in Nebraska. Numbers in bold are statistically significant at $P < 0.05$.

Source	SLA		Needle length		Canopy width		Tree height	
	SS	P	SS	P	SS	P	SS	P
Model	486.7	0.40	48.7	<0.0001	13.7	0.020	61.5	<0.0001
Paternal	236.3	0.23	31.6	<0.0001	6.06	0.036	19.4	0.0002
Maternal	238.7	0.46	7.8	0.20	9.47	0.015	32.36	<0.0001
Individual cross	30.55	0.39	0.10	0.77	1.82	0.075	10.7	0.0004

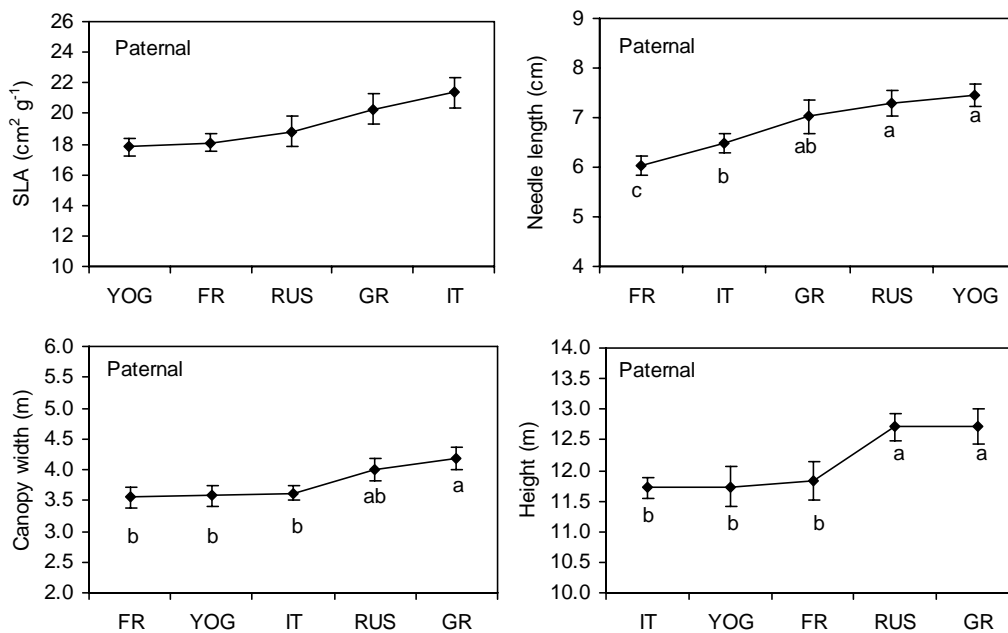


Figure 2. Averages of specific leaf area (SLA $\text{cm}^2 \text{g}^{-1}$), needle length (cm), canopy width (m) and tree height with standard error bars, of a *Pinus sylvestris* progeny plantation in Nebraska, grouped by origin of paternal genetic material (Italy, Greece, France, Russia and Ex-Yugoslavia). Means with similar letters are not statistically different at $P < 0.05$.

Table 2. Orthogonal contrast analysis for specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$), needle length (cm), canopy width (m) and tree height in a Scots pine (*Pinus sylvestris*) progeny plantation in Nebraska. Only significant interactions are presented. Countries were grouped by region of origin (southern European vs. northern European countries). South: SP, GR, and IT; North: RUS, YOG, and GER (FR could be either southern or northern). Numbers in bold are statistically significant at $P < 0.05$. NS = Non Significant.

Source	DF	SLA	Needle length	Canopy width	Tree height
<i>Maternal</i>					
(GR+IT+SP+FR)vs(GER+RUS+YOG)	1	NS	NS	0.023	0.0001
(GR+IT+SP)vs(FR+GER+RUS+YOG)	1	NS	NS	0.037	0.0001
<i>Paternal</i>					
(GR+IT+FR) vs (RUS+YOG)	1	NS	0.0001	NS	NS
(GR+IT) vs (RUS+YOG+FR)	1	0.050	0.0001	NS	0.0001
(GR+IT) vs (FR)	1	NS	0.0001	NS	0.0001
(GR+IT) vs (RUS+YOG)	1	NS	0.0001	NS	0.0001

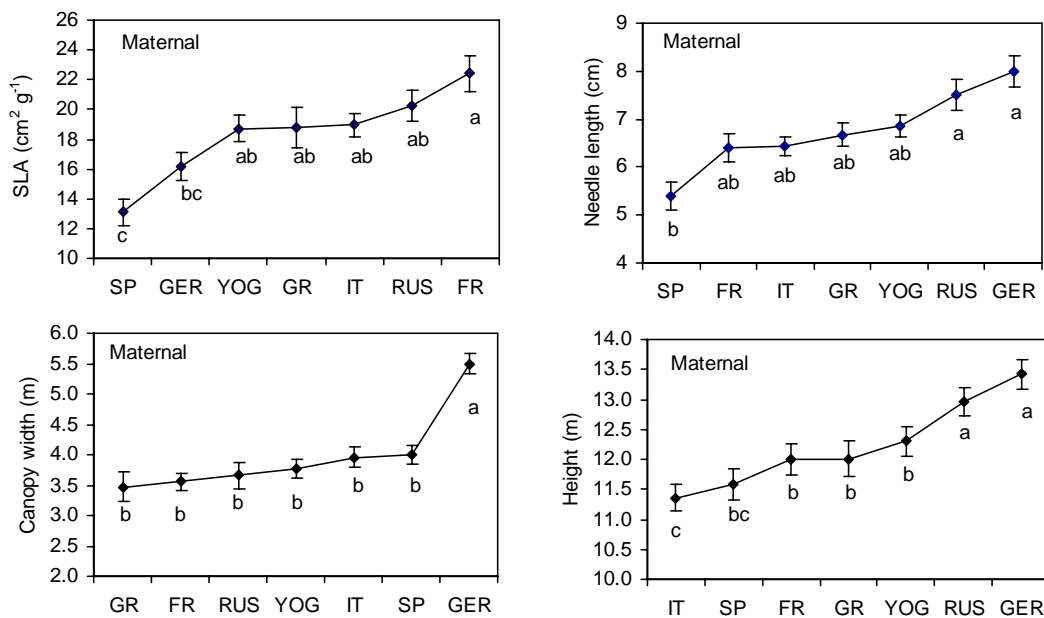


Figure 3. Averages of specific leaf area (SLA $\text{cm}^2 \text{g}^{-1}$), needle length (cm), canopy width (m) and tree height with standard error bars, of a *Pinus sylvestris* progeny plantation in Nebraska, grouped by origin of maternal genetic material (Italy, Greece, Spain, France, Russia, Germany and Ex-Yugoslavia). Means with similar letters are not statistically different at $P < 0.05$.

Table 3. Average specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$), needle length (cm), canopy width (m) and tree height (m), with standard errors (\pm SE), of a *Pinus sylvestris* progeny plantation in Nebraska and representing genetic material from France (FR), Greece (GR), Italy (IT), Russia (RUS), Ex-Yugoslavia (YOG), Spain (SP) and Germany (GER). Means with similar letters are not statistically different at $P < 0.05$.

Paternal	Maternal	n	SLA \pm SE ($\text{cm}^2 \text{g}^{-1}$)	Needle length \pm SE (cm)	Canopy width \pm SE (m)	Height \pm SE (m)
FR	GR	3	17.87 \pm 1.06 d	5.72 \pm 0.16 d	3.00 \pm 0.76 c	11.90 \pm 0.31 c
FR	IT	7	17.72 \pm 0.75 d	5.89 \pm 0.50 c	3.71 \pm 0.42 bc	10.68 \pm 0.40 d
FR	RUS	4	19.68 \pm 0.62 c	6.52 \pm 0.28 c	3.25 \pm 0.14 c	13.12 \pm 0.12 ab
FR	SP	2	12.15 \pm 0.52 e	5.45 \pm 0.08 d	4.00 \pm 0.14 bc	11.59 \pm 0.12 cd
FR	YOG	4	18.81 \pm 1.10 c	6.20 \pm 0.07 c	3.88 \pm 0.30 bc	12.58 \pm 0.59 bc
GR	IT	5	20.63 \pm 0.97 bc	6.28 \pm 0.35 c	4.20 \pm 0.30 bc	11.96 \pm 0.30 c
GR	RUS	3	19.94 \pm 1.69 c	8.64 \pm 0.14 a	4.83 \pm 0.40 ab	14.13 \pm 0.71 a
GR	YOG	3	19.16 \pm 2.50 c	7.77 \pm 0.31 a	4.17 \pm 0.17 bc	13.12 \pm 0.18 ab
IT	FR	6	28.46 \pm 3.01 a	5.74 \pm 0.50 d	3.42 \pm 0.30 c	11.13 \pm 0.34
IT	GER	2	16.18 \pm 1.02 d	8.00 \pm 0.45 a	5.50 \pm 0.30 a	13.42 \pm 0.30 ab
IT	GR	8	19.47 \pm 1.20 c	6.47 \pm 0.35 c	3.38 \pm 0.25 c	11.55 \pm 0.45 cd
IT	IT	6	19.15 \pm 1.75 c	6.11 \pm 0.28 c	4.25 \pm 0.17 bc	11.59 \pm 0.31 cd
IT	RUS	6	22.80 \pm 1.11 b	7.21 \pm 0.61 b	3.50 \pm 0.22 c	12.40 \pm 0.38 bc
IT	YOG	6	18.58 \pm 1.32 cd	6.66 \pm 0.38 b	3.33 \pm 0.11 c	11.69 \pm 0.29 c
RUS	FR	5	18.82 \pm 1.68 c	6.94 \pm 0.57 b	3.70 \pm 0.30 bc	12.75 \pm 0.40 bc
RUS	GR	3	18.98 \pm 0.76 c	7.50 \pm 0.27 b	4.17 \pm 0.70 bc	13.42 \pm 0.35 ab
RUS	IT	2	21.77 \pm 1.72 b	7.98 \pm 0.52 a	4.00 \pm 0.50 bc	11.90 \pm 0.31 c
RUS	RUS	2	13.52 \pm 0.69 e	6.93 \pm 0.51 b	4.50 \pm 0.25 ab	12.81 \pm 0.30 ab
RUS	YOG	2	18.35 \pm 0.68 cd	7.41 \pm 0.69 b	4.25 \pm 0.25 bc	12.35 \pm 0.46 bc
YOG	FR	3	18.27 \pm 0.47 cd	7.20 \pm 0.44 b	3.83 \pm 0.17 bc	12.00 \pm 0.67 bc
YOG	GR	2	17.05 \pm 0.30 d	7.63 \pm 0.29 ab	3.50 \pm 0.50 c	11.90 \pm 0.61 c
YOG	IT	6	17.86 \pm 1.23 d	7.04 \pm 0.21 b	3.75 \pm 0.42 bc	11.23 \pm 0.58 cd
YOG	RUS	2	17.84 \pm 0.89 d	8.91 \pm 0.56 a	2.75 \pm 0.25 c	12.66 \pm 0.15 bc

Multivariate analysis

Results from PCA of measured traits showed that the first three axes were the most biologically significant, explaining around 91% of the total variability (Table 4). Axis 1 (Figure 4), the most important of all, accounted for 49% of the explained variability. Axis 1 differentiated crosses GR x RUS and IT x GER (paternal x maternal) from the remaining crosses (Figure 4). Crosses that scored high on that axis were characterized by the greatest tree height, canopy width and needle length (Table 4). Crosses that scored high for Axis 2 (e.g. IT x FR) had high SLA (accounting 26%, Figure 4). Axis 3 accounted for the least percent of variability and was minor in importance when compared to the other two, explaining 16% of the total variability and appeared to separate mainly IT x FR; IT x GER; IT x IT and GR x IT from the remaining crosses (Figure 4). These crosses were characterized by wide canopy in contrast to YOG x RUS which was characterized by the narrowest.

Table 4. Results from the Principal Component Analysis (PCA) on correlations for all crosses. The total variability explained by the three axes is 91% (axis 1: 49%, axis 2: 26% and axis 3: 16%).

	Axis 1	Axis 2	Axis 3
Specific leaf area	-0.241	0.841	0.485
Needle length	0.552	0.393	-0.411
Canopy width	0.507	-0.299	0.768
Height	0.616	0.224	-0.073

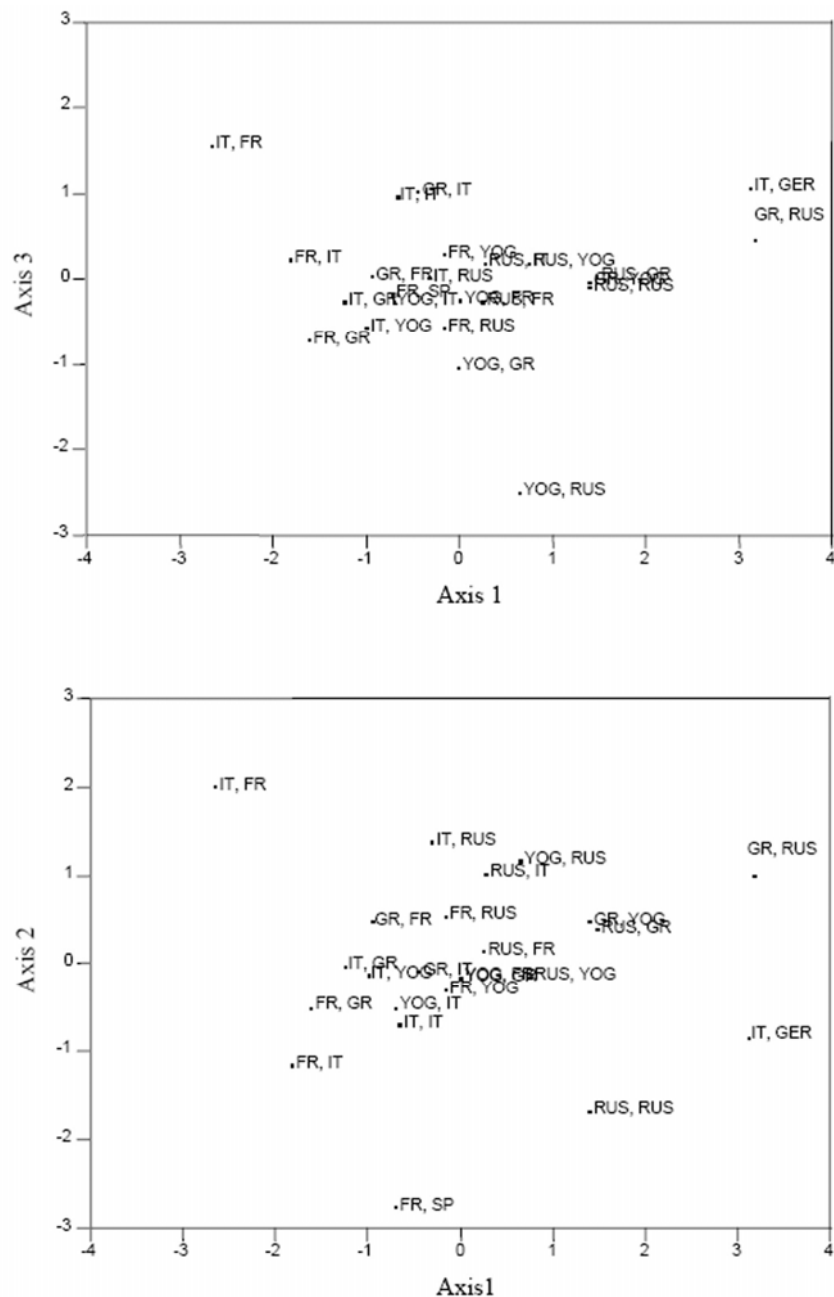


Figure 4. Crosses (paternal, maternal) scores on Axes 1, 2 and 3 from the Principal Component Analysis of individuals from a *Pinus sylvestris* progeny plantation in Nebraska and representing genetic material from France (FR), Greece (GR), Italy (IT), Russia (RUS), Ex-Yugoslavia (YUG), Spain (SP) and Germany (GER).

4. Discussion and Conclusions

This study aimed at evaluating a twenty-two-year-old *Pinus sylvestris* plantation of progeny selected from crosses from seven known European provenance origins. The SLA, needle length, canopy width, and tree height of each of the 23 individual crosses were measured and data was correlated with the seed source to identify the source(s) that are potentially most adapted to the Great Plains environment.

Analyses of variance and principle components indicated that height, followed by canopy width, and needle length, were the most reliable predicting variables. Similarly, other studies have shown that tree height is a key indicator of vigor and is under strong genetic control (Mátyás 1997). Maternal sources from northern latitudes performed better than southern sources. Axis 1 from the PCA showed that trees with maternal genetic material from Russia and Germany performed better than other sources, and were characterized by the greatest tree height, needle length and canopy width (the later for Germany, Figure 4). These are all important factors in forest and windbreak managements and for Christmas tree plantations. Similarly, Van Haverbeke (1986) found that seed sources from Germany performed better (grew faster) than other European sources in Eastern Nebraska.

Height is one of the easiest and fastest measurements of vigor that can be taken in the field, allowing it to be used by producers and non-scientists in the future as an inexpensive and simple method of testing. Height of dominant trees can also be used to estimate forest site productivity (Korkalainen 2005), which becomes important to producers who not only need to determine vigor of certain trees but also for the determination of site index.

There is widespread support for regional genetic differences in Scots pine populations in Europe. However, there is not a consensus on where lines should be drawn. There are several factors that influence and contribute to the genetic diversity in a species, and the emergence of genetically distinct ecotypes. These include climatic conditions, photoperiod, latitude, altitude, length of the growing season, seed migration, fragmentation, and isolation and drift (Mátyás 1997, Oleksyn et al. 2001). This study found significant differences between southern (Spain, Greece, and Italy) and northern sources (Russia, Ex-Yugoslavia, and Germany). France was more or less in between and might be classified as central. Similarly, Oleksyn et al. (2001) reported the existence of three distinct populations and separated them by latitude: northern ($>58^{\circ}\text{N}$), central ($55\text{--}48^{\circ}\text{N}$), and southern ($<45^{\circ}\text{N}$). Oleksyn et al. (2000) found that southern sources of Scots pine

experienced high mortality rates when transferred north, possibly due to increased vulnerability to diseases and other biotic and abiotic stresses.

The maternal sources of Russia and Germany performed best when crossed with paternal sources from Greece and Italy, respectively (Fig. 4). The maternal sources might have provided the genetic tolerance for the continental winter in the Great Plains (low winter temperatures) and paternal sources might have provided the ability to withstand the relatively dry and hot summers. Further molecular and physiological testing might provide better answers. In general, results of this study have shown that maternal seed sources from northern Europe (Russia, Ex-Yugoslavia, and Germany) are better adapted to the Great Plains environment than southern sources. These sources will be best suited for windbreaks, Christmas trees, and landscaping.

The original intent of the Scots pine improvement programs, which included this progeny test, was to find the best sources of seed stock for Christmas tree plantations. The same factors that made the trees desirable as Christmas trees also made them ideal for planting at shelterbelts and windbreaks. The future goals of this progeny test were: 1) evaluation of the select trees through progeny testing, 2) recurrent selection from within these progenies, and 3) their conversion to either an improved seedling seed orchard or the establishment of a second production clonal seed orchard (Van Haverbeke 1976). However, due to the seriousness of PWN, current research is focusing on the identification and development of crosses that are disease resistant. Various levels of success have been reported to increase resistance of pine to PWN with either breeding or grafting (Toda and Kurinobu 2001, Masanori and Toda 2002, Fei and Heli 2004), or to prevent infection by using insecticides/nematicides such as abamectin (James et al. 2006). Lastly, it is worth noting that the Scots pine plantation used in this study has recently been infected by PWN and efforts are being made to remove infested trees and control the spread of the disease.

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